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Further, it is unclear whether Aoyama intended for the luteolin to be administered orally, at the claimed dosage rate. If anything, Aoyama made a presumption, not supported by any valid experimental evidence or publication.

In the scientific discipline, making these kinds of presumptions and assumptions, such as on the basis of Aoyama's teachings, based on separate and unrelated *in vitro* experiments, is very common. However, these assumptions have to be evaluated by *in vivo* experimental conditions in the intact body, particularly with respect to complicated disorders such as asthma, which result from effects on a plurality of different biological pathways.

In vitro experiments of evaluating any compound show its effect on only one or a few parameters. In the body, thousands or more biological reactions are going on. A particular agent, that affects only one or a few parameters *in vitro*, may affect many other additional parameters in the body, which may or may not be favorable to alleviate the targeted disease. The agent will be active against the disease only when it really affects that particular targeted disease in the intact body. In many cases, it has been found that such generalized presumptions and assumptions were shown to be absolutely opposite to that of the experimental findings.

Accordingly, at best, Aoyama might have suggested performing additional research on luteolin, but did not disclose the claimed invention. Taken as a whole, luteolin was noted as one of the least active compounds, and as such, it would not have even been obvious to focus on luteolin over apigenin or the other compounds disclosed as being more active than luteolin.

Further, Aoyama has observed the histamine inhibiting activity of the extract. However, the existing literature suggests that all histamine release inhibitors are not necessarily anti-asthmatic agents. For example, as reported by Bousquet et al (1992) "Histamine is an important mediator of asthma. The new anti-histamines, which block histamine at the H1-receptor level also possess some anti-allergic properties. At current dosages they appear to be safe. These drugs are effective in the treatment of rhinitis and conjunctivitis but their role in asthma is still under investigation. At a dose higher than usually recommended, they were shown to block exercise-induced asthma and, inconstantly, the early phase reaction after allergen challenge. Their effect on the late phase allergic reaction as well as their clinical efficacy during trials is, however, less consistent. The indication of H1-blockers in the treatment of asthma is therefore limited, especially since doses higher than recommended may lead to adverse reactions".

In light of the above points, Aoyama, which only disclosed *in vitro* tests, does not explicitly disclose the anti-asthmatic activity of luteolin in the intact body. Therefore, Aoyama's teachings merely extrapolate a theorized effect, but fail to provide a sufficient showing to anticipate the claimed invention.

Accordingly, Aoyama fails to teach the claimed invention, and Applicants respectfully request that the novelty rejection be withdrawn.

Claims 1, 2, 4-8, and 10-13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Wang. This rejection is respectfully traversed.

First, Applicants respectfully assert that bronchitis is not the same as asthma (Blewase and Raymon, 2002). Wang experimented on patients with bronchitis, not patients with asthma. He did not define any clear anti-asthmatic activity of luteolin. For example, Wang did not disclose evaluating luteolin on a single characteristic feature of asthma, such as EAR, LAR, or even on the biochemical parameters such as histamine release, IL-4, IL-5, IFN- α and IGE in the serum or bronchoalveolar lavage fluid.

Therefore, the assertion that the functional effects for preventing the development of asthmatic features of LAR AND EAR, and of increasing IFN- α to a normal level and decreasing each of IL-4, IL-5, and IGE to a normal level, as well as inhibiting airway constriction and airway hyperreactivity are inherent to the method of treatment taught by Wang is an extrapolation at best. Applicants respectfully assert that Wang does not anticipate the claimed subject matter.

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Murai et al.

Murai tested certain lipoxygenase inhibitors such as luteolin and demonstrated some anti-allergic activity in the ear, but did not teach any method of treating asthma. As discussed above, not all anti-allergic agents are anti-asthmatic agents, and this could not have been confirmed without testing in an asthma model. It would not have been clear that luteolin had anti-asthmatic properties without *in vivo* testing.

The Examiner relies on the principle of inherency by stating that, since Murai administered the same drug at the same concentration, it inherently would have treated any

asthma that would have been present in any of the children. Applicants respectfully assert that the Examiner has misapplied the principles of inherency. That lutcolin would treat asthma is not taught or suggested by Murai. If the Examiner's application of the principles of inherency were applied generally, there would be no claims available for new methods of using old drugs.

Accordingly, Murai fails to teach the claimed invention, and the rejection should be withdrawn.

Claims 1, 2, 4-8 and 10, 12, and 13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Kotani et al. This rejection is respectfully traversed.

Kotani teaches that flavonoid compounds fisetin, quercetin, luteolin, and others are suitable for treating and preventing allergic diseases. Asthma is not specifically listed. Kotani did not test any compound in a living animal, only in *in vitro* experiments. As discussed above, the mere measurement of *in vitro* data is not sufficient in this instance to anticipate the claimed invention. Indeed, quercetin, one of the mentioned flavonoid compounds, has no anti-asthmatic effect in mice, but rather, made the animals very sick and led to their death. Therefore, although quercetin is a known PLA2 inhibitor and a strong anti-inflammatory flavonoid (Lee et al. 1982; Lanni and Becker 1985), it has an adverse effect and reduced the life span (Jones and Hughes, 1982).

In conclusion, Kotani fails to teach or suggest that lutcolin is useful for treating asthma at all, let alone in the claimed mode of administration and dosage rate. Accordingly, Kotani does not anticipate the claimed invention.

Rejections under 35 U.S.C. 103 (a)

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 103 (a) as obvious over Murai in view of Tanaka and Nagai. This rejection is respectfully traversed.

Inventiveness of the Claimed Methods

Inventiveness lies in the new idea and the new method of carrying it out, when the idea has led to a new and advantageous result. Here, the new idea relates to the use of a plant-based, non-toxic compound in extremely low doses in an animal model of asthma, where lutcolin

exhibited preventive and curative anti-asthmatic effects, before and after asthmatic manifestation in the animals.

While the method may appear very simple and obvious using impermissible hindsight, it is not so. The Applicants have carried out well-formulated laboratory experiments to elucidate the anti-asthmatic properties of luteolin. These experiments involved several steps, including sensitization with an antigen; development and measurement of allergen-induced airway constriction, EAR and LAR along the associated parameters of increased levels of IL-4, IL-5 and IgE, and decreased levels of interferon- α in the serum and bronchoalveolar lavage fluid (BALF). These characteristics were developed in a mouse model and luteolin was tested during the stages of development, as well as after the development of the asthmatic features, to evaluate its preventive and curative anti-asthmatic effect. The art made of record is silent regarding any such experiments.

There has been a long-felt need for finding a non-steroidal drug with negligible side effects for treating and/or preventing asthma. It is evident from the prior art that the problem had remained without a solution for a long time, and the claimed invention provides a solution to the problem.

Applicants have found that luteolin, a plant based compound, possesses anti- asthmatic activity (both preventive and curative) in intact, conscious, spontaneously-breathing mice, without exhibiting side effects. A good, working animal model, and not *in vitro* or *ex vivo* isolated systems, is required for testing the efficacy and toxicity of a candidate drug in an asthma model. If the activity of a candidate drug is tested on deceased animals or in isolated organs such as the trachea, it is not possible to observe and determine both the efficacy and toxicity of the compound, because the pharmacological effects of a drug is not confined to a single isolated system.

In the scientific literatures drawing this kind of extrapolation or assumption from far-fetched in-vitro experiments from cell culture and tissue from dead body is very common. Because *in vitro* experiments of evaluating any compound show its effect on only one or few tested parameters. In the body thousands and more biological reactions are going on. The agent that affects only a few parameters or biochemicals *in vitro* may affect many other additional parameters also in the body that may or may not be favourable to alleviate the targeted disease.

The agent will be anti-disease only when it really alleviates that particular targeted disease in the intact living body. Moreover, any inhibitor of inflammatory enzymes is not anti-asthmatic. For example, cyclooxygenases (COXs) are enzymes which cause severe inflammation in the body, but their inhibitors are not anti-asthmatic; and not only this, they aggravate asthma and instructed not to give bronchosensitive/ asthmatric patients (Namazy and Simon, 2002, Simon, 2004).

Analysis of the Cited References

Murai has been discussed above.

Nagai disclosed using a medicinal preparation, including several compounds, to treat dermatitis in the ears of mice, and investigated the effect of luteolin on histamine release from mast cells. It is not known how much activity was contributed by which constituent component. Further, while histamine release may be associated with many diseases, histamine release inhibitors used in any other system or *in vitro* are not necessary antiasthmatic. For example, histamine antagonists clemastine, ketotifen, and azelastine have no significant effect on methacholine-induced bronchoconstriction in asthmatics (Nogrady et al. 1978; Albazzaz and Patel; 1988 Cockcroft 1992).

Tanaka was cited for teaching that luteolin inhibits the activity of hexosaminidase release from mast cells, inhibits the release of histamine from mast cells or basophils, and suppresses cysteinyl leukotriene synthesis.

Applicants note that the Examiner has extrapolated the fact that luteolin was shown to have certain effects on certain biochemical pathways as rendering obvious the use of luteolin for all disorders that have been associated with a similar biochemical pathway. As discussed in detal below, this is not the case.

This logic seems to imply that all that needs to be shown to satisfy an obviousness rejection is that a drug has an affect on any relevant pathway that is ultimately associated with treating a particular disorder, even if treatment of the particular disorder was not taught. This is not, and should not be, the law, particularly with respect to disorders mediated by multiple biochemical pathways.

In conclusion, the combined teachings of these references do not render obvious the claimed invention.

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 103 (a) as obvious over Kotani in view of Tanaka and Nagai. This rejection is respectfully traversed.

As with the prior obviousness rejection, Kotani does not teach a method of treating asthma. The same comments presented above apply to this rejection as well.

Conclusion

On the basis of the arguments raised herein, Applicants respectfully assert that the invention as claimed is novel and non-obvious over the cited art. Withdrawal of the pending rejections, and allowance of the claims, is respectfully requested. The Examiner is encouraged to contact the undersigned if any questions remain.

Respectfully submitted,

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Docket No.: C261 1030.1

Appendix- Listing of the Claims

1. (Previously Presented) A method of preventing and/or treating asthma in animals including humans using natural compound Luteolin, said method comprising administering a composition consisting essentially of a therapeutically effective dose of the Luteolin to the animal,

wherein the Luteolin is administered orally, and

wherein the Luteolin is administered in an amount in a range of 0.1 to 10 mg/kg of body weight of the animal.

2. (Previously Presented) A method as claimed in claim 1, wherein the compound Luteolin shows negligible side effects.

3. (Cancelled)

4. (Previously Presented) A method as claimed in claim 1, wherein the method prevents development of asthmatic features comprising Early Airway Response (EAR) and Late Airway Response (LAR).

5. (Original) A method as claimed in claim 1, wherein level of IFN-gamma increases to normal level.

6. (Original) A method as claimed in claim 1, wherein level of IL-5 decreases to normal level.

7. (Original) A method as claimed in claim 1, wherein level of IL-4 decreases to normal level.

8. (Original) A method as claimed in claim 1, wherein level of IgE decreases to normal level.

9. (Cancelled)

10. (Previously Presented) A method as claimed in claim 1, wherein Luteolin is administered in an amount in a range of 1 to 10 mg/kg of body weight of the animal.

11. (Previously Presented) A method as claimed in claim 1, wherein Luteolin is administered to the animal for a time period in a range of 5 to 10 days.

12. (Previously Presented) A method as claimed in claim 1, wherein the Luteolin inhibits airway constriction.

13. (Previously Presented) A method as claimed in claim 1, wherein the Lutcolin inhibits airway hyperactivity.

Appendix B - Cited References

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